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Unless unforeseen difficulties arise, the Illinois State Academy of Science will hold its thirty-seventh annual meeting on May 5 and 6, 1944, at DeKalb, Illinois, with the Northern Illinois State Teachers College acting as host. Dr. H. W. Gould, professor of chemistry at that institution, will act as chairman of the committee on local arrangements and already has his committee for the next year's meeting well organized and at work.

L. R. TEHON,
Secretary

SPECIAL ARTICLES

ISOLATION AND PROPERTIES OF CHICKEN ERYTHROCYTE NUCLEI[1]

LASKOWSKI recently has published an improved method for the isolation of undamaged chicken erythrocyte nuclei.[2]. This method is undoubtedly much superior to the previously existing methods, but a disadvantage of the procedure is that lysolecithin must be used, which is not a convenient substance to prepare and which might not be desirable in case lipid studies were to be made with the isolated nuclei.

We have found that if washed chicken erythrocytes are laked in 0.9 per cent. sodium chloride by means of saponin, the nuclei are rapidly liberated and can be readily isolated by centrifugation followed by washing in 0.9 per cent. sodium chloride solution. The concentration of the sodium chloride solution is very important, since for example if one uses 0.85 per cent. instead of 0.9 per cent. sodium chloride, the nuclei almost invariably form a gel as the result of agglutination while they are being washed.

Our procedure was as follows: Fresh chicken blood was collected at the slaughter house and was defibrinated. The erythrocytes were centrifuged and were washed in 0.9 per cent. sodium chloride solution according to the procedure of Laskowski,[2] except that we have washed only twice. Then they were suspended in sufficient 0.9 per cent. sodium chloride solution to make a volume equal to that of the original volume of blood. To one hundred cc of erythrocyte suspensions was added 5 cc of 0.11 molar phosphate buffer of pH 6.8 to 7.0 containing 0.3 gm of Merck's purified saponin. Practically complete laking occurred in five minutes. The nuclei were then washed four or five times with 0.9 per cent. sodium chloride solution. Usually we have added two or three cc of 0.11 molar phosphate buffer of pH 6.8 to 7.0 to the centrifuged nuclei without stirring, immediately before adding the sodium chloride solution.

Nuclei prepared by this method were found to be of even better microscopic appearance than those prepared by us according to the method of Laskowski. However, we used snake venom to prepare our lysolecithin instead of bee venom as employed by Laskowski. Although upon first examination nuclei prepared using lysolecithin or saponin appeared to be free from stroma, heavily stained preparations examined under high power were found to contain a certain amount of extremely tenuous stroma attached to the nuclei. It appears possible that complete removal of this stroma causes agglutination of the nuclei.

The total lipid content of our nuclei is about 14 per cent., of which a considerable fraction appears to be phospholipid. The desoxyribonucleic acid content of the nuclei, determined by the Dische reaction[3] according to the procedure of Seibert[4] is in the neighborhood of 45 per cent.

The respiration of the nuclei without added substrate was compared with the respiration of nuclei prepared by the method of Laskowski,[2] and also with that of nuclei prepared at pH 6.0 by laking with very dilute citric acid. In the latter preparation much stroma appeared to remain attached to the nuclei,

[1] From the Department of Biochemistry and Pharmacology, University of Rochester School of Medicine and Dentistry, Strong Memorial Hospital, Rochester, New York.

[2] M. Laskowski, *Proc. Soc. Exp. Biol. and Med.*, 49: 354, 1942.

[3] Dische, *Z. Mikrochemie*, 8: 4, 1930.

[4] F. B. Seibert, *Jour. Biol. Chem.*, 133: 593, 1940.

making it impossible to wash out the last traces of hemoglobin. The QO_2 values for these preparations are given in Table 1.

TABLE I

	Nuclei prepared using saponin	Nuclei prepared by Laskowski's method	Nuclei prepared by C citric acid method
QO_2 in 0.9% NaCl buffered to pH 7.4 with phosphate, 37.5° C.	0.40	0.10 ±*	0.10 ±
QO_2 in 0.11 molar phosphate, pH 6.8, 25° C.	0.26	...	...

* Laskowski reports an average value of about 0.20 for the QO_2 of his nuclei, with a variation of 0.15 to 0.22. We have made only one determination in duplicate on Laskowski nuclei and our sample may have stood a trifle too long in the ice box before testing.

Nuclei prepared by our method were rather intensely yellow from a pigment which we believe to be largely xanthophyll. To study this pigment the nuclei were washed once with 95 per cent. alcohol, and this washing was discarded. Then the nuclei were extracted three times with 95 per cent. alcohol and the extracts, which contained nearly all the pigment, were combined. The alcohol was evaporated to dryness, leaving a residue of the pigment together with some lipid. This residue was taken up in a small volume of hot alcohol, and on cooling some of the lipid precipitated and was removed. The concentration of alcohol then was lowered to about 92 to 93 per cent. by the addition of water and the pigment was removed from the alcohol by repeated extractions with petroleum ether. These petroleum ether extracts were combined. Subsequent extraction of the petroleum ether with a small volume of 92 per cent. methanol caused the pigment to pass almost quantitatively from the petroleum ether into the methanol layer. The methanol was evaporated to dryness and the pigment, which still contained some lipid, was taken up in carbon disulfide. The carbon disulfide solution gave absorption bands at 444.5, 476.0 and 507.5 mμ, which coincide almost exactly with the positions of bands reported in the literature for xanthophyll.[5] The pigment gave two observable bands in petroleum ether at 444.0 and 475.5 mμ, and in 92 per cent. methanol two bands at 448.0 and 478.0 mμ could be seen. A sample of the pigment that had been evaporated to dryness gave a strong blue color with the Carr-Price reagent.

Although the original alcoholic extract containing the yellow pigment showed practically no fluorescence, a sample of nuclei in which the xanthophyll had gradually disappeared during storage yielded, after drying, a light yellow ether-alcohol extract which was appreciably fluorescent, possibly indicating a flavin pigment.

When the isolated nuclei were suspended in distilled water and the pH was adjusted to about 8.5 with ammonia, a transparent apparently structureless gel was formed which persisted in extreme dilutions. The transparency of this gel is of interest in view of the considerable amount of lipid present. The gel is coagulated to form a rather fibrous precipitate by the addition of a little dilute acetic acid. A similar gel is also formed if 5 or 10 per cent. sodium chloride is added to the centrifuged nuclei, and Laskowski states that saturated sodium chloride solution also causes gel formation. It appears likely that the gel must represent some sort of heavily hydrated lipo-nucleoprotein complex with the lipid in a fairly high degree of dispersion.

SUMMARY

(1) A method has been described for the isolation of chicken erythrocyte nuclei using saponin to lake the cells. Nuclei prepared by this method have been compared with nuclei prepared by the method of Laskowski[2] and with nuclei prepared by a citric acid method, particularly in regard to respiration.

(2) The total lipid content of the nuclei is about 14 per cent. and the desoxyribonucleic acid content is about 45 per cent.

(3) A yellow pigment contained in the nuclei appears to be xanthophyll.

(4) A gel formed by the nuclei at pH 8.5 in the presence of ammonia and the absence of other cations is described.

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ALEXANDER L. DOUNCE
TIEN HO LAN

FRACTIONAL CEPHALIN-CHOLESTEROL FLOCCULATION IN HEPATIC DISEASE[1]

SINCE 1938, when Hanger[2] first described the ability of sera from patients with intrahepatic disease to flocculate an emulsion of cephalin and cholesterol, a number of reports have appeared in the literature (summarized in a recent contribution from this institution[3]) indicating the validity of this laboratory procedure as an aid in the study of disorders of the liver. This test has proved to be of particular value in the differentiation between hepatogenous jaundice and

[1] From the Medical Research Laboratory and the Division of Pathological Chemistry, New York Post-Graduate Medical School and Hospital, Columbia University, New York.

[2] F. M. Hanger, *Trans. Asn. Am. Physicians,* 53: 148, 1938; *Jour. Clin. Invest.,* 18: 261, 1939.

[3] C. H. Greene and M. Bruger, *N. Y. State Jour. Med.,* 43: 318, 1943.

[5] H. Gilman, "Organic Chemistry, An Advanced Treatise," Vol. II, page 1149. New York: John Wiley and Sons, 1938.